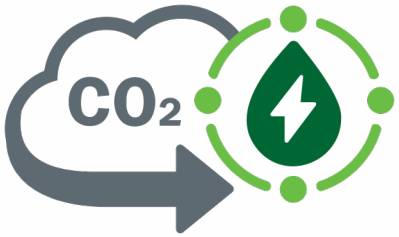


CO_2 Reduction and Upgrading for e-Fuels Consortium

U.S. DEPARTMENT OF ENERGY

NREL

BETO 2023 Peer Review

CO_2 Valorization via Rewiring Carbon Metabolic Network

April 7, 2023

Principal Investigator: Wei Xiong

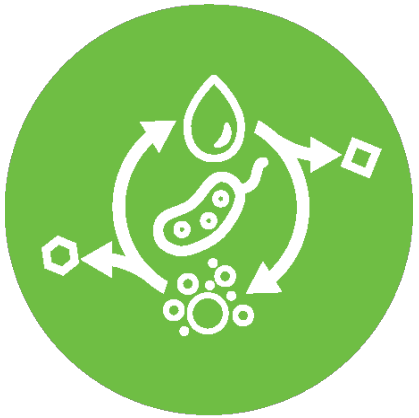
Technology Area Session: CO_2 Utilization

Organization: National Renewable Energy Laboratory

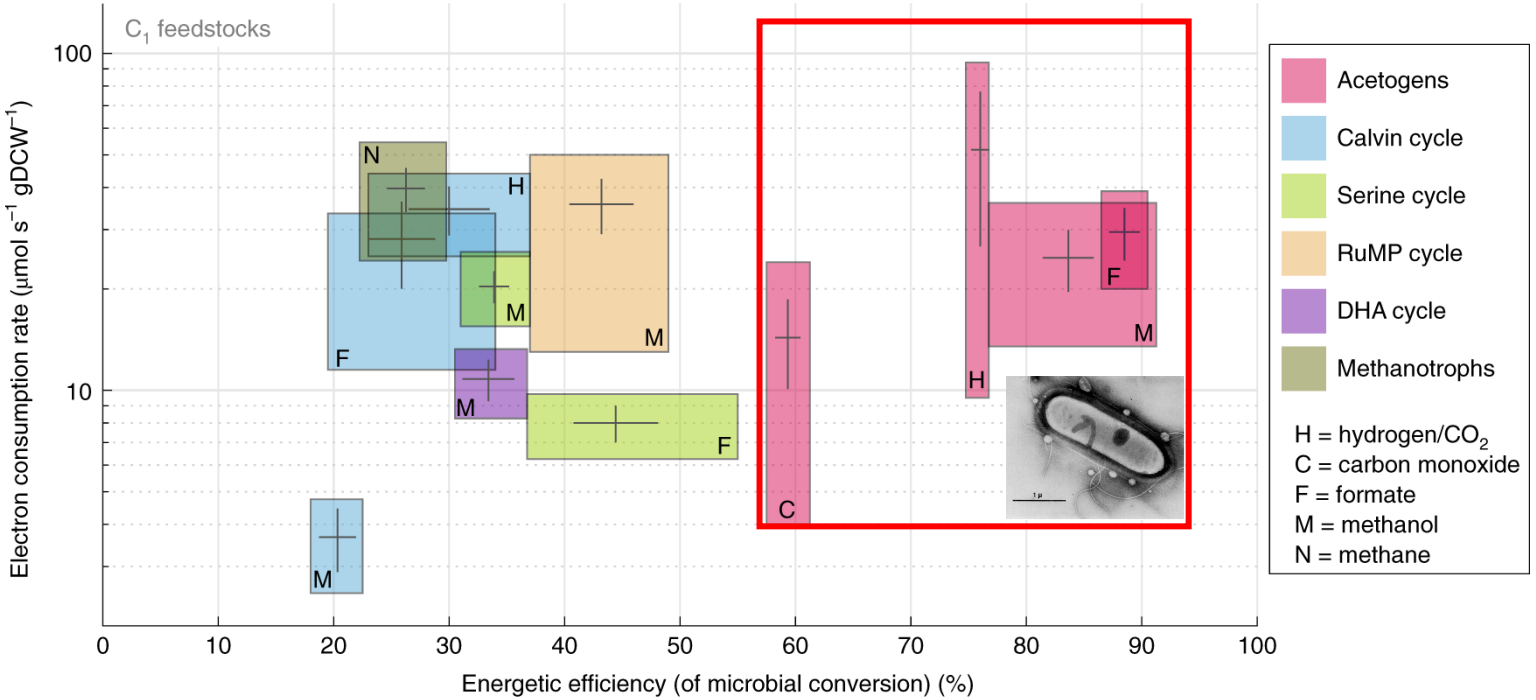
Project Overview

Developing Carbon Negative Biorefinery with Gas-fermenting Bacteria

- Acetogens: Model gas-fermenting anaerobes
- High energy efficiency and fast electron/carbon consumption ($CO/CO_2/H_2$/Formate/Methanol)
- Pathway diversity (the CO_2-fixing Wood-Ljungdahl pathway and glycolysis) AND redesign
- To develop a new chassis for the production of biofuels and value-added chemicals



Biological Upgrading



Claassens et al. ***Nature Catalysis***. 2019, 2: 437–447

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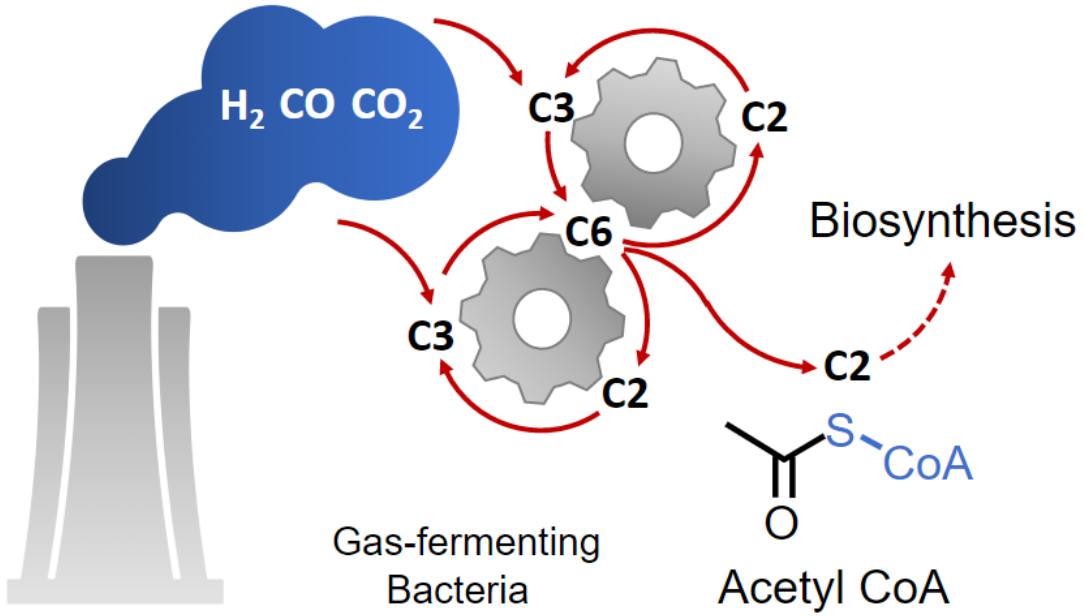
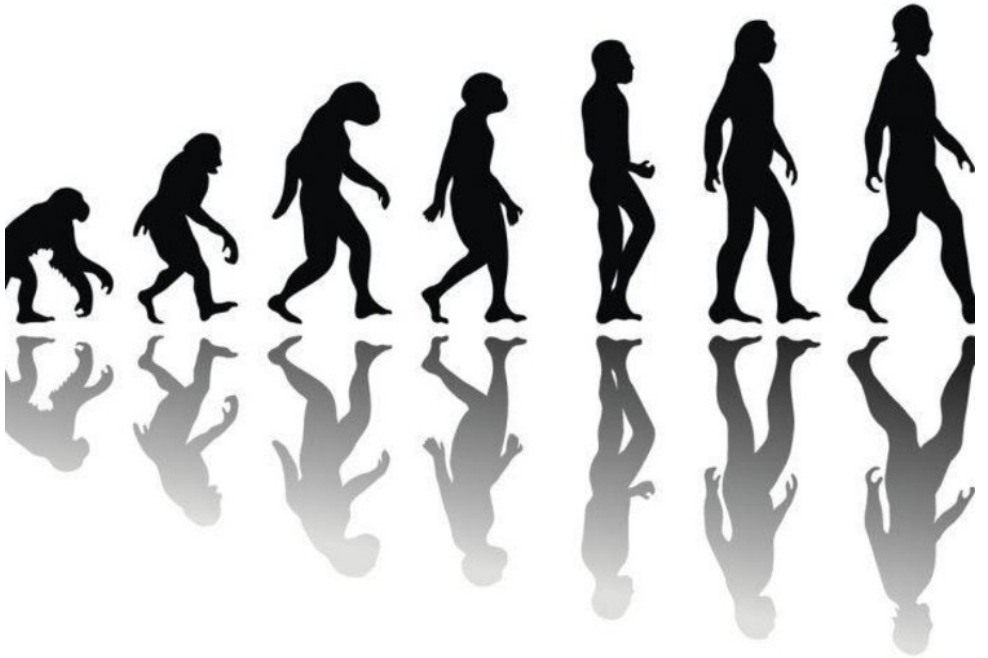


Wu , Lo and Xiong*, et al. ***Nature Synthesis***. 2022, 1, 615–625

1 Approach – Strain Engineering and Optimization

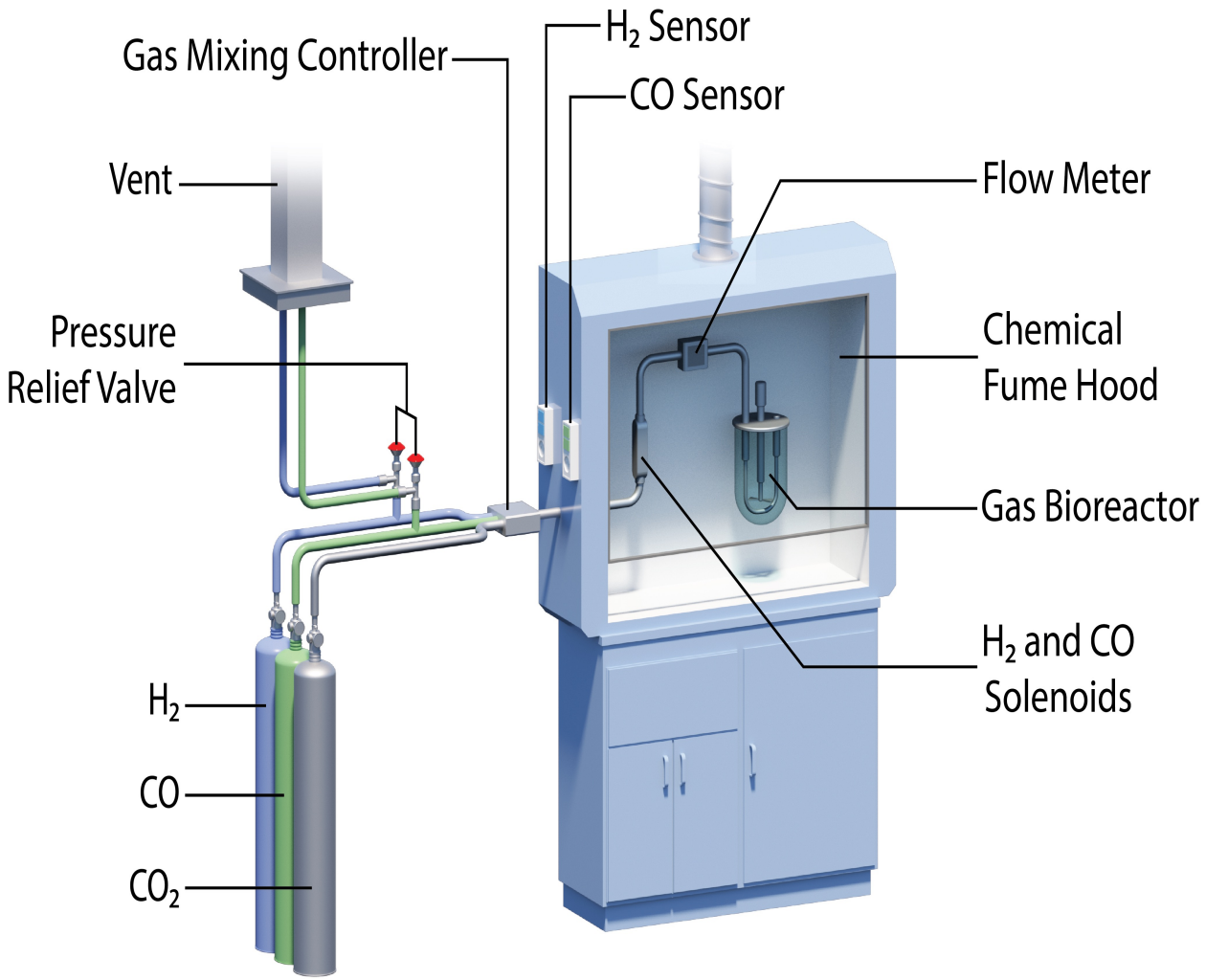
CRISPR-cas9 genome editing



Adaptive laboratory evolution

Lo, Humphreys, Xiong, et al. ***Front Bioeng and Biotech***. 8, 560726

1 Approach – Lab Scale Syngas Fermentation



Gas fermentation and safety setup with controllable mixing of syngas

1 Approach – Bioinformatics Guided Rational Engineering

Pathway Dissection and Design

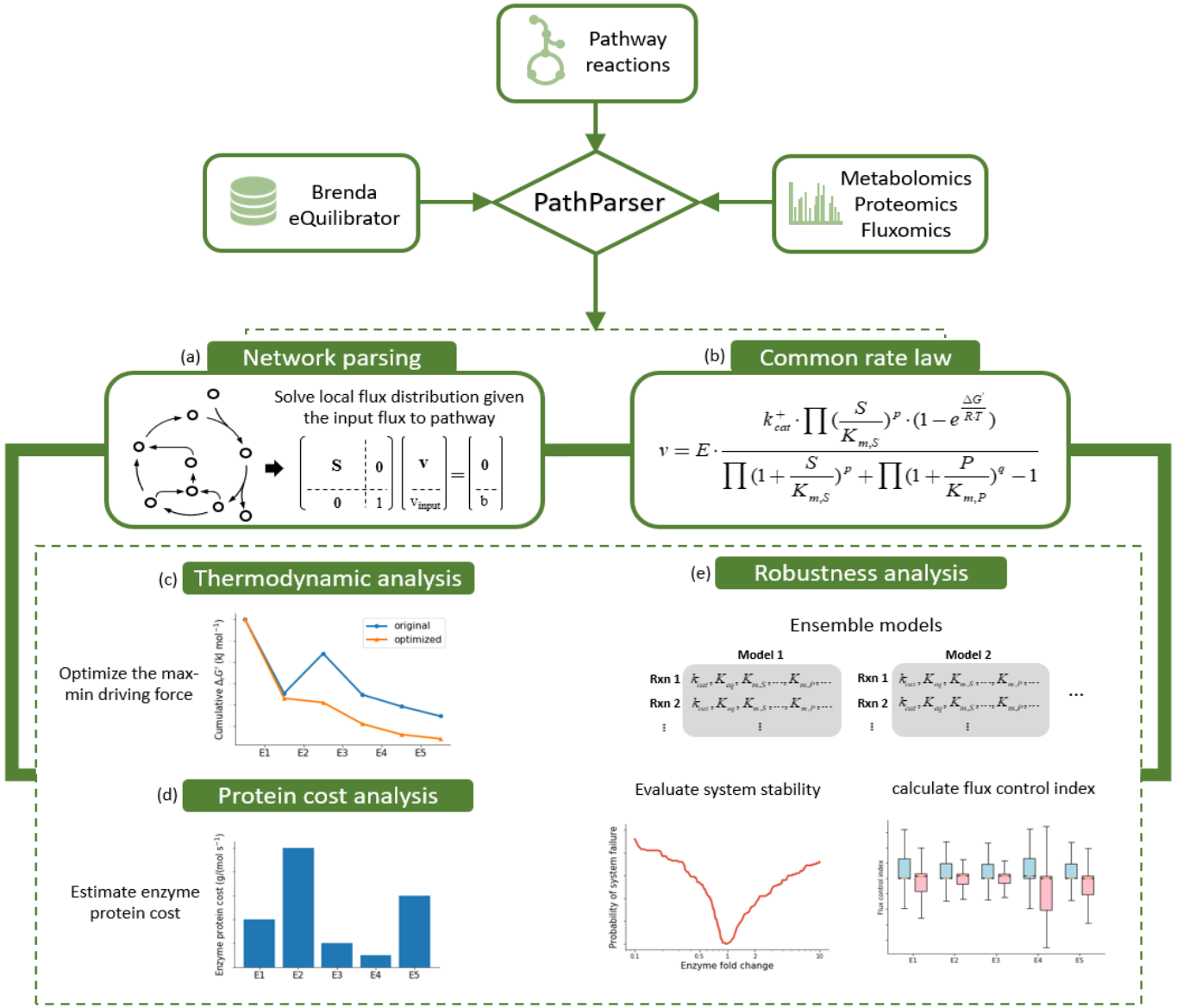
Q: Pathway feasibility
A: Thermodynamic Optimization

Q: Pathway expense
A: Protein Cost Analysis

Q: Ability to resist perturbation
A: Metabolic Robustness Analysis

Rational Engineering

- Predict and Identify potential engineering targets
- Provide actionable insights into strain engineering for optimal bioproduction



Wu et al. ***Metabolic Engineering***. 2020,57,140-150
Xiong* et al. ***mSystems***. 2023 (In press)

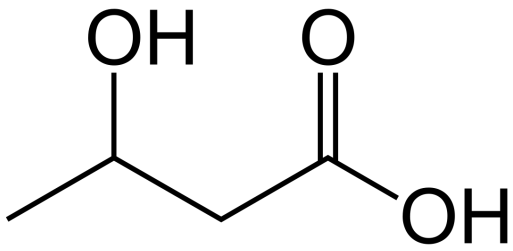
2 Progress- 3-Hydroxybutyrate Production from Syngas

Developing Gas-fermenting Chassis for 3HB Production from CO_2

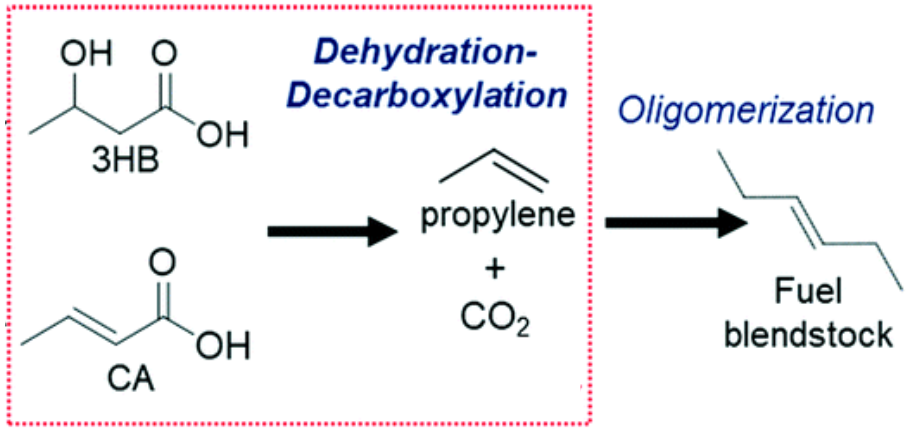
- A platform compound for fine chemicals, medicines, and biofuels
- The precursor to biodegradable plastics
- Upgradable to propylene and hydrocarbon fuel blendstocks
- The production of 3HB from CO_2 has been investigated in photosynthetic cyanobacteria by us, but with inherent limitations that are barriers to industrial application.

  Wang and Xiong et al. ***Green Chemistry***. 2018, 20 (16), 3772-3782

- This project aims at non-photosynthetic CO_2 valorization to 3HB



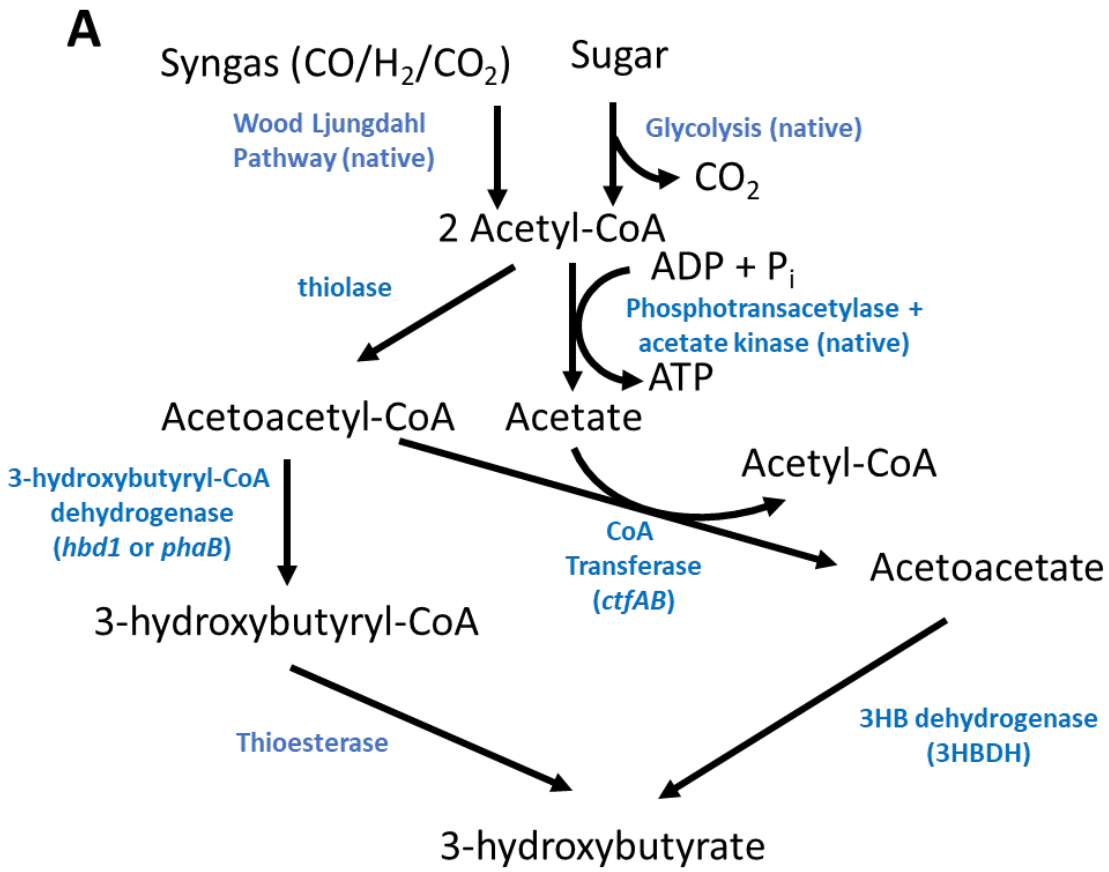
3-Hydroxybutyric Acid

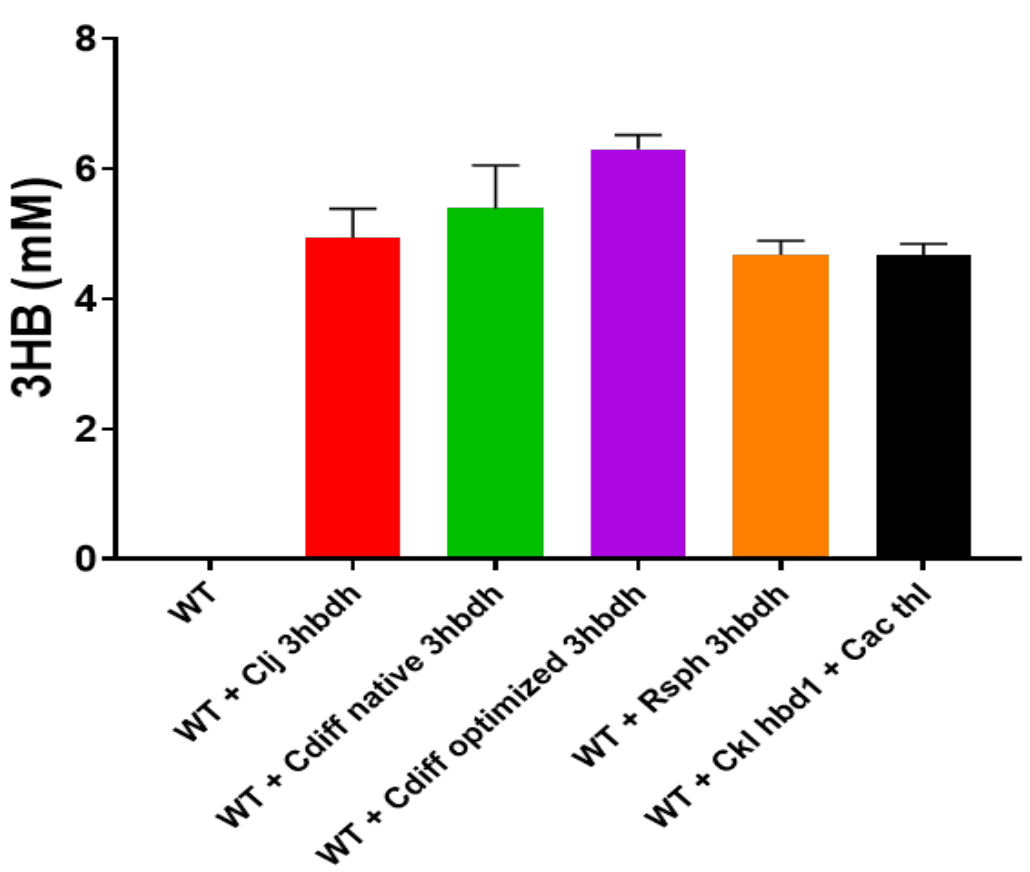


Integrated processing for conversion of 3HB to propylene and renewable hydrocarbon fuel blendstocks

Leow et al. ***Catal. Sci. Technol***., 2021, 11, 6866-6876

2 Progress- Pathway Design and Engineering



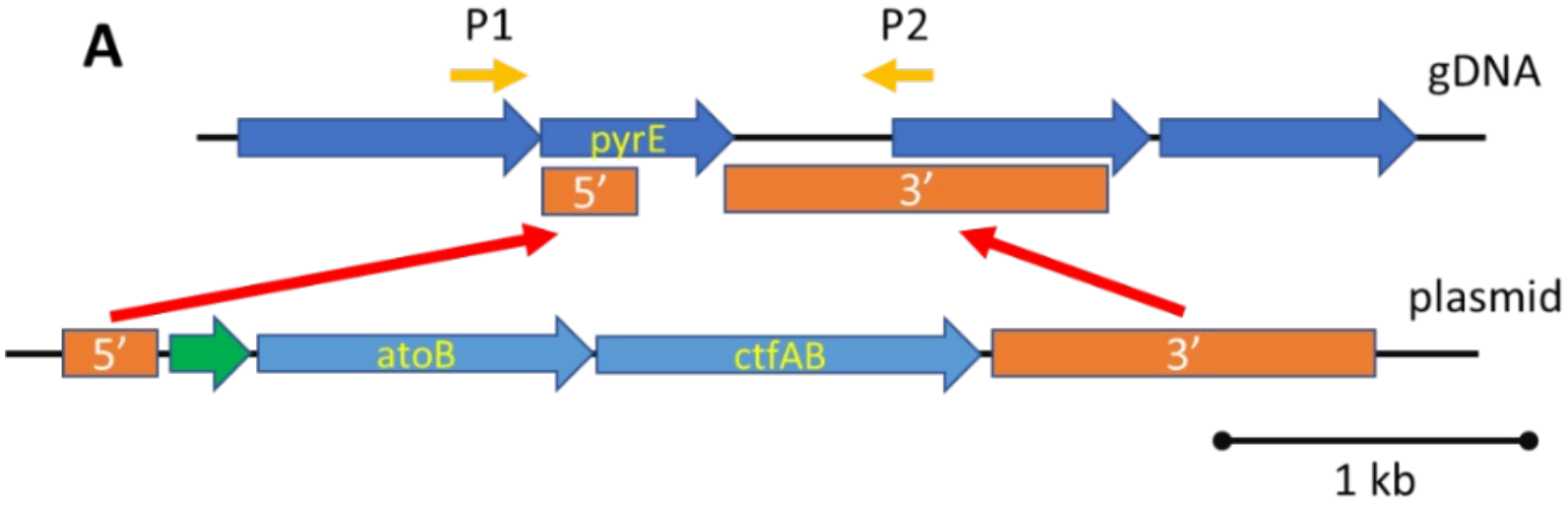


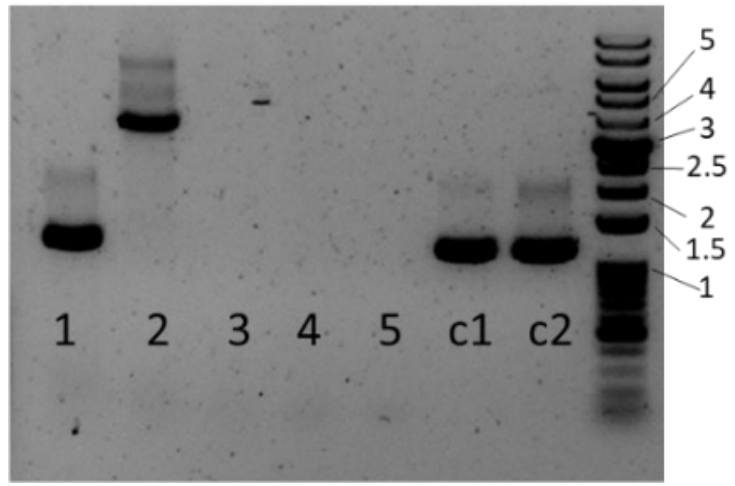
Developing Gas-fermenting Chassis for 3HB Production

- Heterologous expression of key pathway genes leads to 3HB production in *C. ljungdahlii*

Lo, Humphreys, Xiong et al. ***Frontiers in Microbiology***. 2022:948369

2 Progress- Genome Integration of Selected Pathway Genes



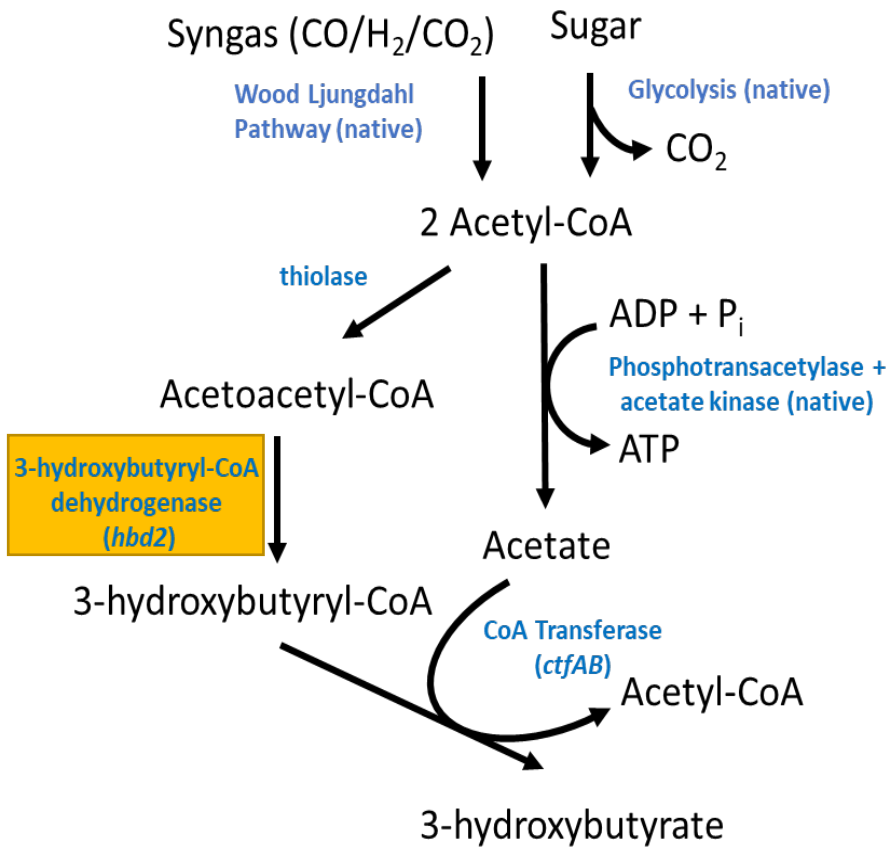


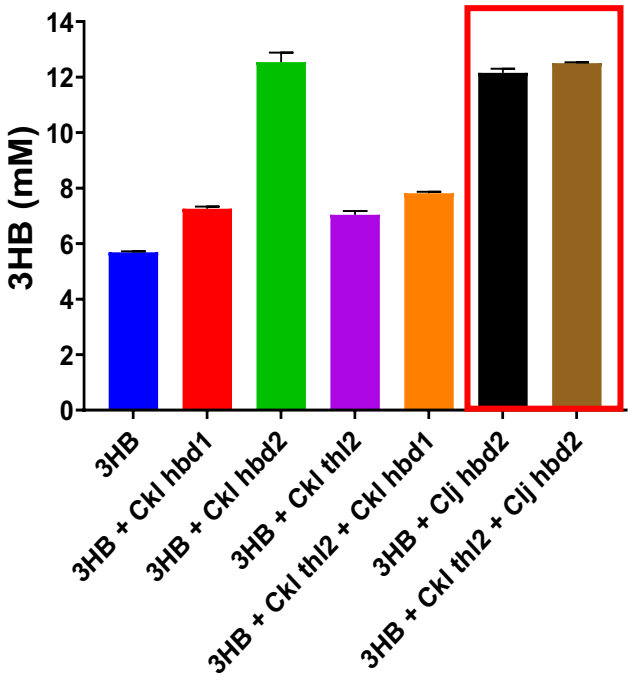
Developing Gas-fermenting Chassis for 3HB Production

- Integration of selected pathway genes into the neutral site of the genome
- Integration strains demonstrated improvement in genetic stability
- Genome Integration allowed iterative strain engineering

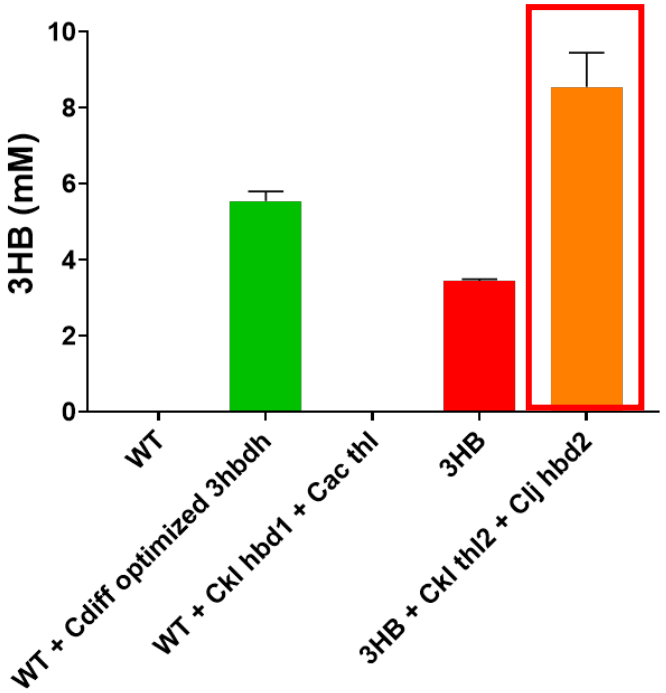
Lo, Humphreys, Xiong et al. ***Frontiers in Microbiology***. 2022:948369

2 Progress- Native 3HB Pathway to Enhance 3HB Production





+ Fructose



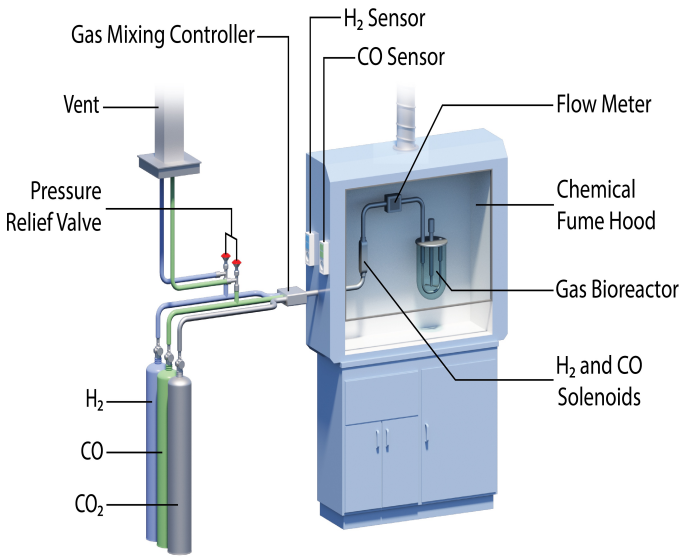
+ Syngas ($CO/CO_2/H_2$:70/20/10)

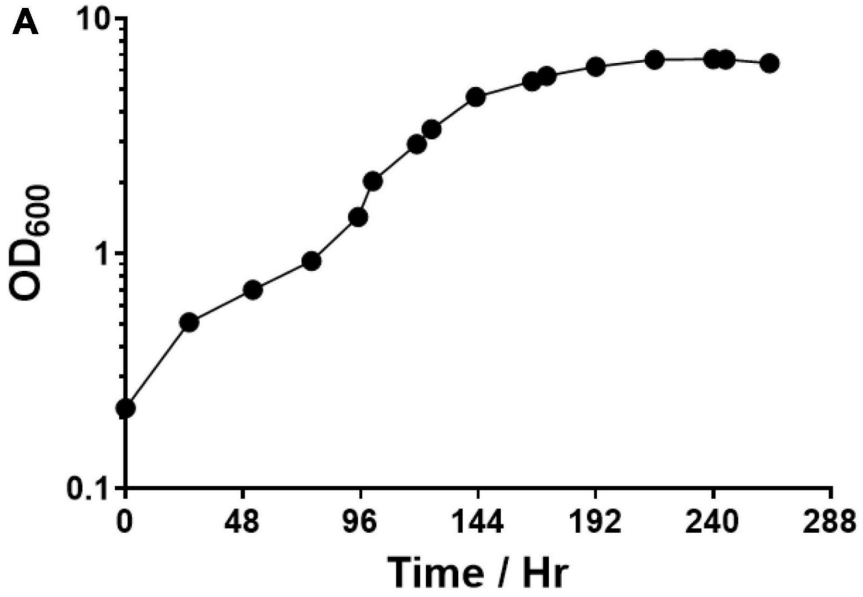
Developing Gas-fermenting Chassis for 3HB Production

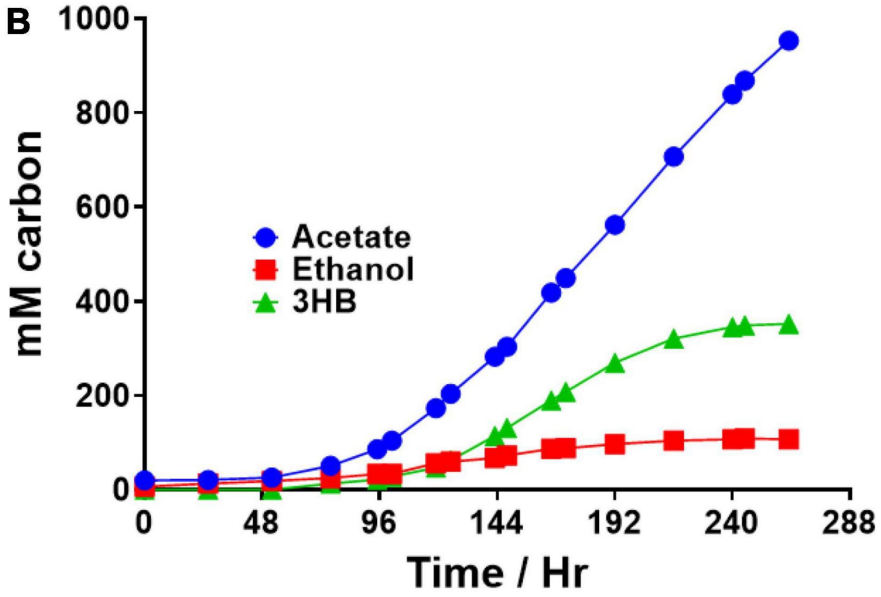
- Identification of a hbd2 gene in *C. ljungdahlii*
- Overexpression of this gene in the 3HB parental strain increased 3HB titer

Lo, Humphreys, Xiong et al. ***Frontiers in Microbiology***. 2022:948369

2 Progress- High-Titer 3HB Production in Gas-fermenting Reactor





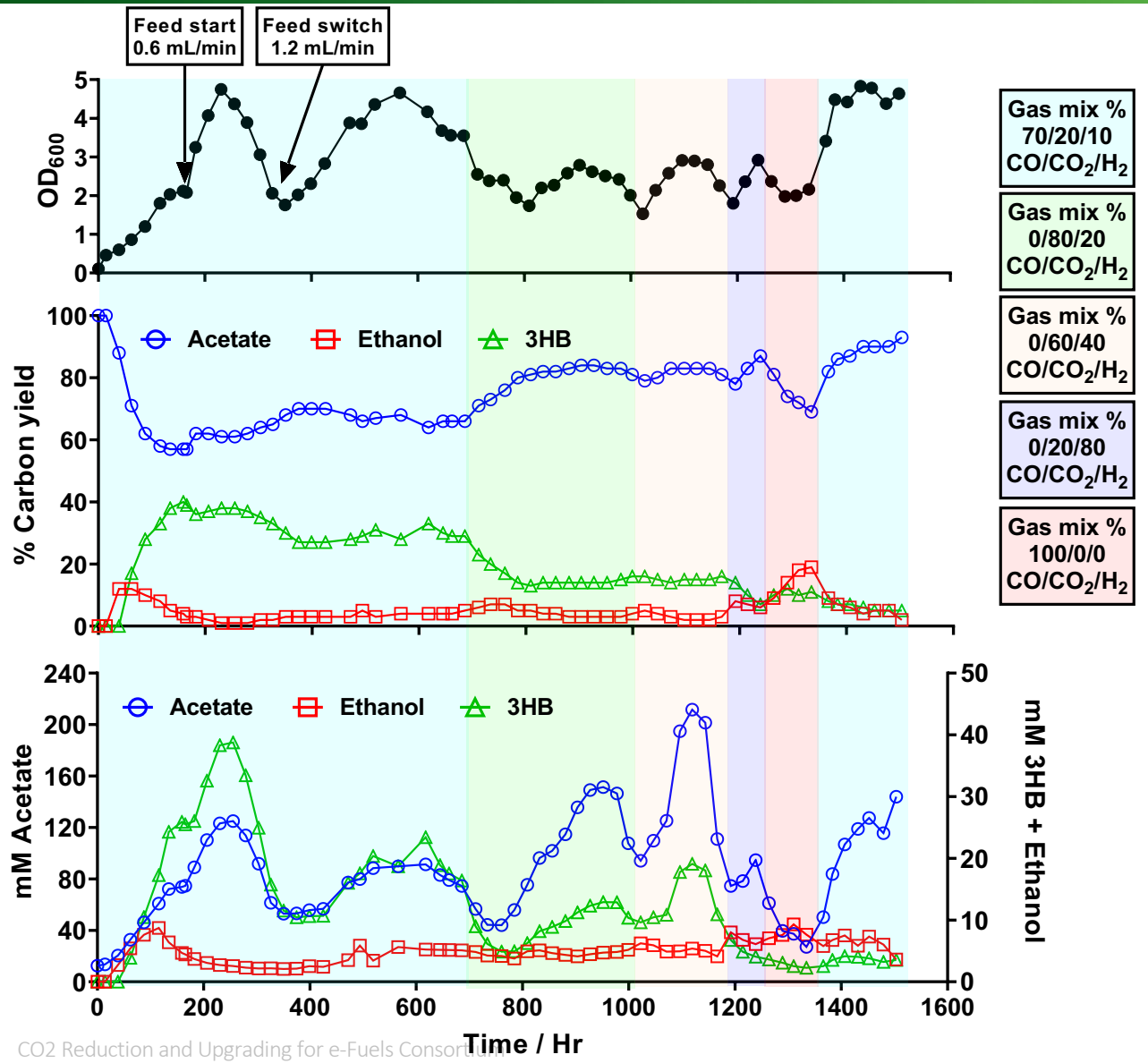


Developing Gas-fermenting Process for 3HB Production

- 9.15 g/L titer and ~0.1 g/L/hr maximum production rate achieved for 3HB production.
- Per **Techno-Economic Analysis (TEA)**, the productivity is approaching industrial requirement.

Lo, Humphreys, Xiong et al. ***Frontiers in Microbiology***. 2022:948369

2 Progress- Continuous 3HB Production in Gas-fermenting Reactor

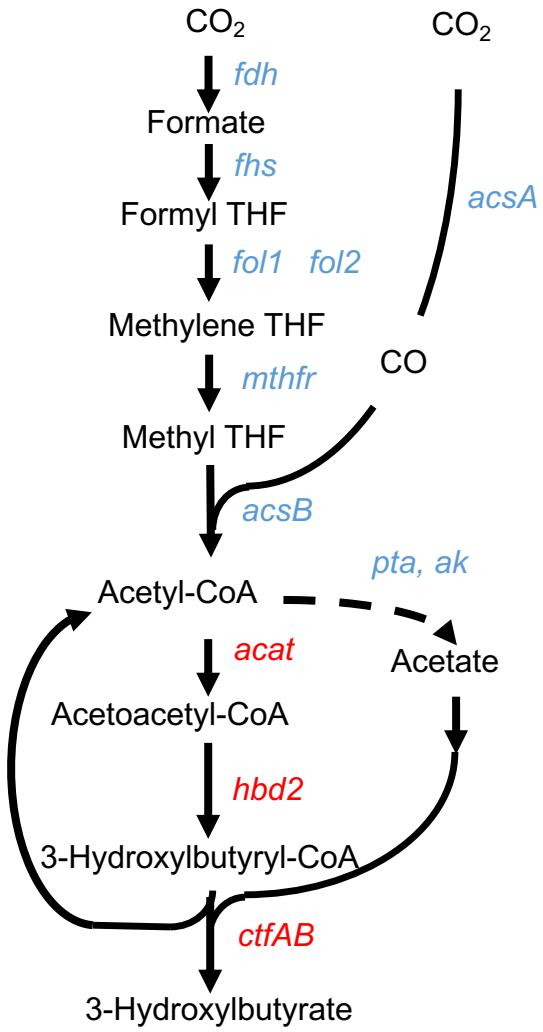


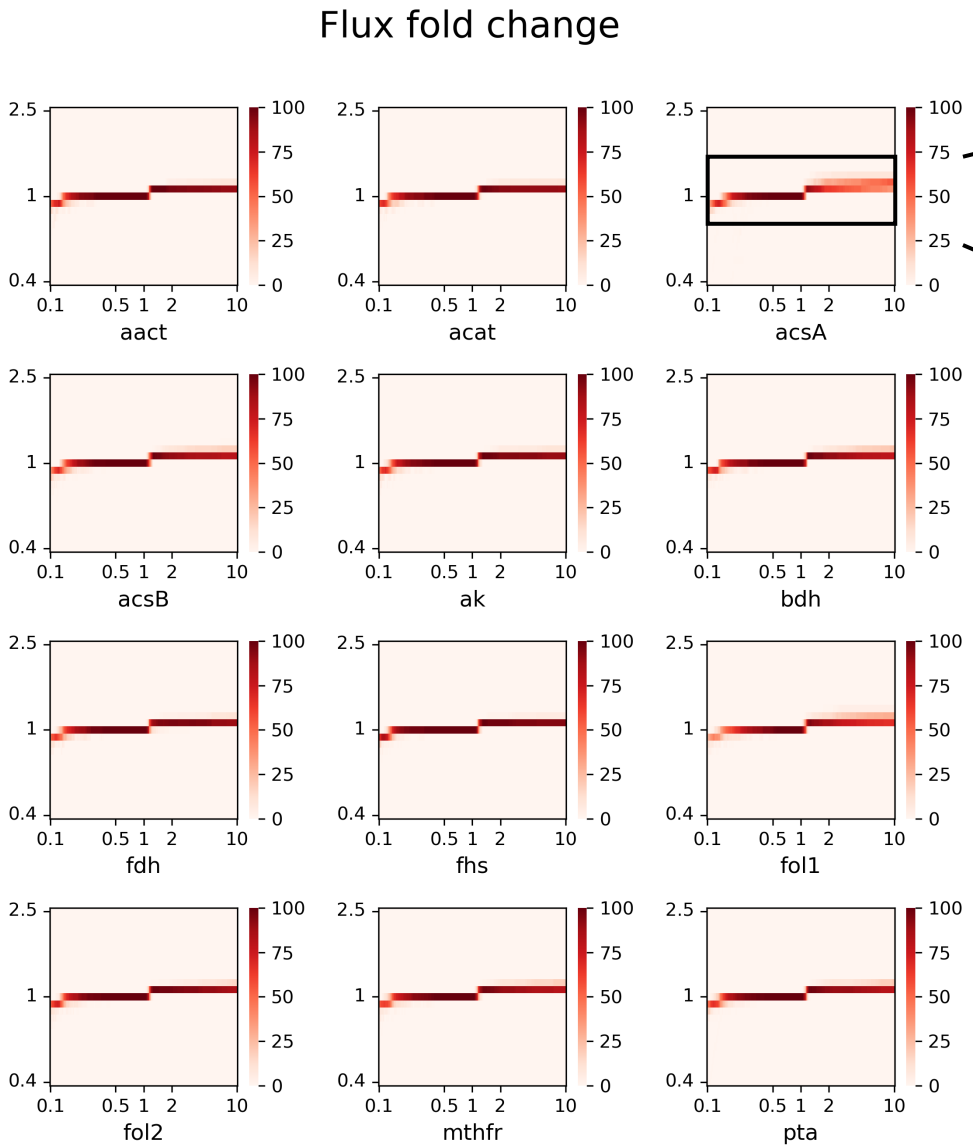
3HB production sustained in continuous fermentation for more than 50 days!

Milestones for FY23

Milestone Name/Description	Criteria	End Date	Type
Thermodynamic and kinetic modelling that assess the effect of improved hydrogen utilization.	Report outcomes from thermodynamic and kinetic models that predict thermodynamic feasibility and enzyme protein requirement for a H^2-utilizing clostridial pathway.	12/31/22	QPM
Non-native hydrogenase expression in *Clostridium ljungdahlii*	Transform and express a non-native hydrogenase in *C. ljungdahlii* and report the effects on growth andS product formation.	3/30/23	QPM
Adaptive laboratory evolution (ALE) for improved carbon-utilizing strains	Run an ALE experiment either in pressure bottles or using a continuous system and isolate adapted acetogen strains.	6/30/23	QPM
Improve carbon conversion efficiency in commercial acetogen strains.	Using our 3HB producing strain, produce a titer of 12 g/L of 3HB which represents at least 30% of the carbon yield from syngas using our 2 L bioreactor system.	09/30/23	Annual SMART milestone

2 Ongoing- Identify Potential Targets for Optimal Production





Identification of flux control coefficients (FCCs)

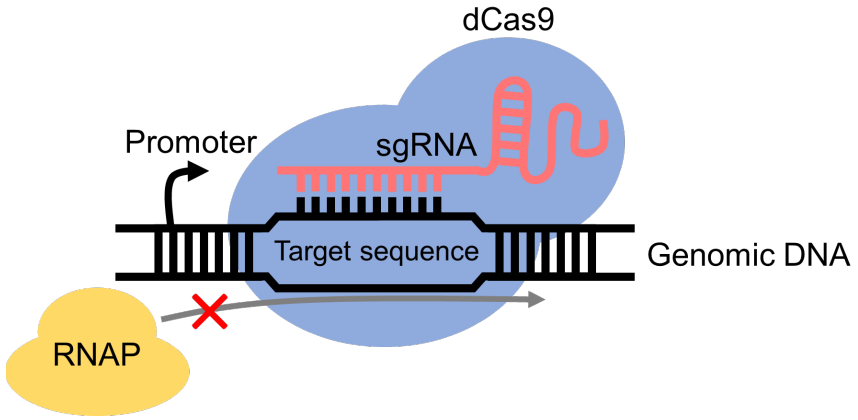
- The ensemble modeling yields the steady-state metabolite and flux solutions corresponding to kinetic parameter perturbations.
- The solutions of the ensemble can guide the selection of potential targets for improving performance.

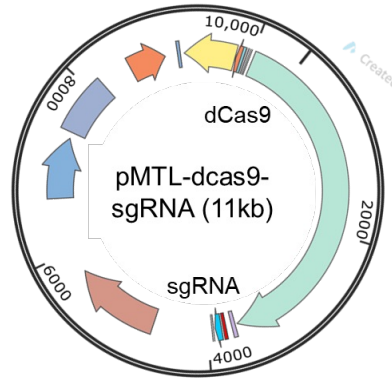
2 Ongoing- CRISPRi of the Target Gene Improved 3HB Production

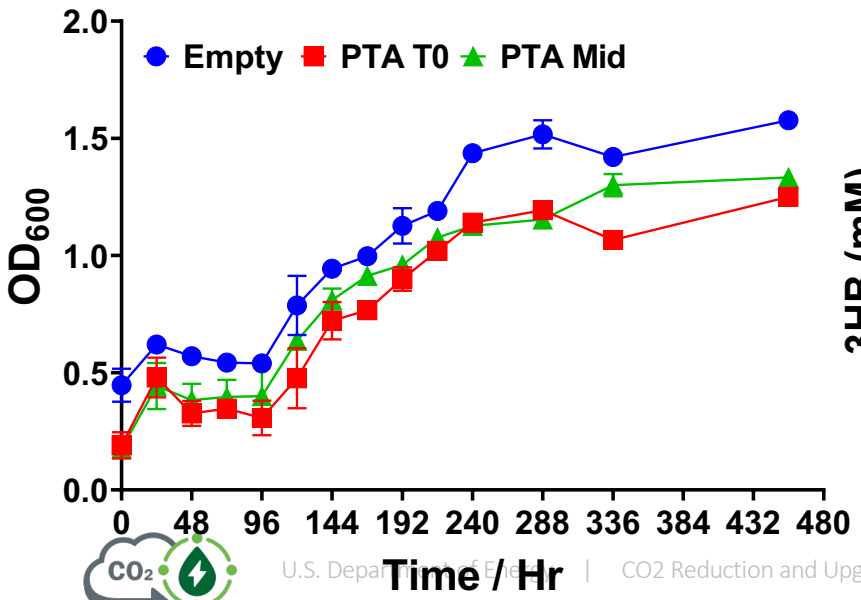
Improving 3HB production by CRISPR interference

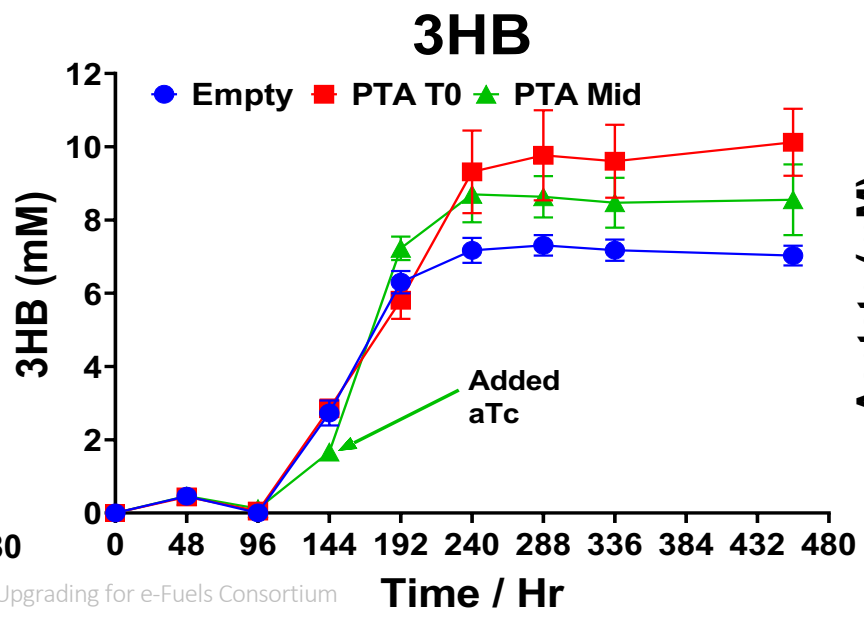
- Development of CRISPRi in a gas-fermenting chassis enabled rapid, iterative strain engineering
- CRISPRi targeting the *pta* gene (encoding phosphate acetyltransferase) has increased 3HB titer as well as decreased acetate titer from a competing pathway

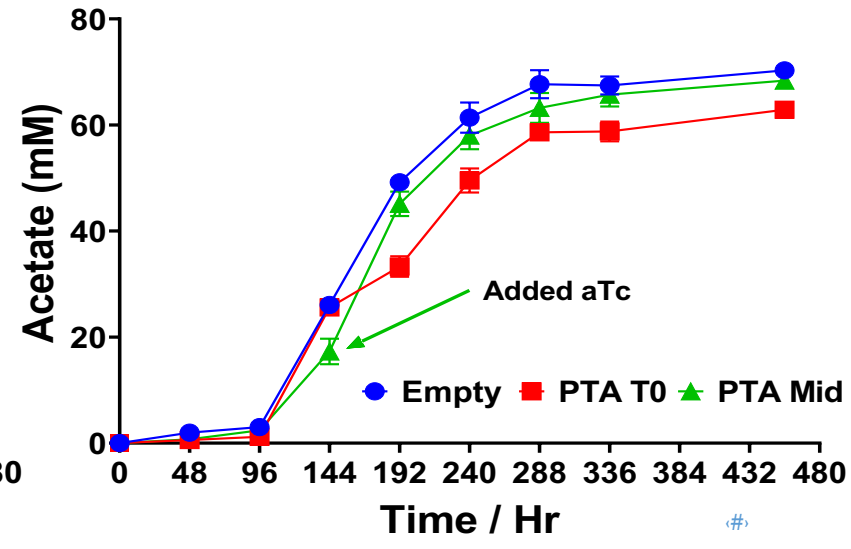
CRISPR Interference











3-Impact

Industrial Application

- Developing strains and processes that can improve industrial scale production of 3HB as well as other valuable chemicals (*i.e.* acetate and ethanol) using *C. ljungdahlii*
  - We have engaged with two companies (*i.e.* Shell) for collaborations on gas-fermentation technology and/or 3HB production.
  - One patent application has been generated from this work.
    - Jonathan Lo, Jonathan Humphreys, Wei Xiong. Genetically engineered bacterium for the production of 3-hydroxybutyrate. **Patent Application No. PCT/US23/61553**

Scientific

- Strengthening R&D synergies with other teams and industrial partners (*i.e.* Lanza Tech) in the CO_2RUe consortium
- Developing cutting edge approaches for fast, iterative and rational engineering of gas-fermenting hosts
- Understanding thermodynamics and kinetics of processes for biological syngas valorization
- One research article has been published and we anticipate additional 1-2 from this work.
  - Jonathan Lo, Jonathan R Humphreys, Lauren Magnusson, Benton Wachter, Chris Urban, Wei Xiong, Katherine J Chou, Pin Ching Maness. Acetogenic production of 3-Hydroxybutyrate using a native 3-Hydroxybutyrate-CoA Dehydrogenase. **Frontiers in Microbiology**. 2022; 13: 948369.

Quad Chart Overview

Timeline

- *10/01/2020*
- *9/30/2023*

	FY22 Costed	Total Award
DOE Funding	*$376,155*	*$1,000,000*
Project Cost Share *		

TRL at Project Start: *2*
TRL at Project End: *4-5*

Project Goal

This project aims to develop a new carbon-based economy by capturing CO_2 and valorizing it to the high-value products in an autotrophic microbe that can fix CO_2 non-photosynthetically.

End of Project Milestone

Improve hydrogen utilization and carbon conversion efficacy in commercial acetogen strains. Show a >10% increase in hydrogen utilization from isolated strains.

Funding Mechanism

This project started as an AOP in 2020, and then incorporated into CO_2 reduction and upgrading for e-FUELs consortium.

Project Partners*

Synergistic within the CO_2RUe Consortium

Summary

Key Accomplishments

- Designed and constructed 3HB pathways in a model gas-fermenting bacterium.
- Identified a native 3HB pathway in the host.
- Achieved conversion of syngas to 3HB through strain development.
- Improved conversion of syngas to 3HB through rational and iterative strain development.
- Achieved industry-relevant production rate and titer in gas bioreactor using CO_2/CO/H_2 as sole carbon source.
- Sustained continuous gas fermentation for 3HB production in lab-scale bioreactor for more than 50 days.

Acknowledgements & Questions

Thank You

NREL

- Jonathan Humphreys
- Jonathan Lo
- Chao Wu
- Chris Urban
- Michael Resch

BETO

- Ian Rowe

CO_2 Reduction and Upgrading for e-Fuels Consortium

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